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Serial No.: 09/731,255
Page 2

Please delete the paragraph at the top of page 4 (lines 1-4).

Please replace the paragraphs at p. 19 describing Figure 8 with the following.

a2

Figure 8A-E depicts expression of the neural stem cell-specific marker nestin in a distinct cell population within pancreatic islets as determined by immunocytochemistry or RT-PCR.

Please replace the paragraphs at p. 19 describing Figure 9 with the following.

a3

Figure 9A-C depicts characterization of nestin in stem cells isolated from the pancreas by immunocytochemistry and RT-PCR.

Please replace the paragraphs at p. 19 describing Figure 10 with the following.

a4

Figure 10A-D depicts expression of homeodomain protein IDX-1 and proglucagon in human islet-like clusters derived from nestin-positive islet progenitor cells (NIPs).

Please replace the paragraphs at p. 19 describing Figure 11 with the following.

a5

Figure 11A-C demonstrates localization of nestin-positive cells to localized regions of the ducts of the rat pancreas.

Please replace the paragraphs at p. 19 describing Figure 15 with the following.

ab

Figure 15A-C depicts expression of neuroendocrine, exocrine pancreatic and hepatic markers in human NIP cultures containing stem cells.

Please replace the paragraph at page 18, lines 21 through 23, with the following replacement paragraph:

a7

Figure 2 shows the result of RT-PCR performed using mRNA obtained from 50 rat islets. Forward and reverse primers are indicated. The depicted primer sequences are: forward primer GCGGGGCGGTGCGTGACTAC (SEQ ID No: 3) and reverse primer GGGTGGTGAGGGTTGAGGTTTGTG (SEQ ID No: 55). The single band of 834 bp was sequenced and identified substantially as the sequence for nestin.

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Please replace the paragraph at page 19, line 3, with the following replacement paragraph:

a8 ~~Figure 7 depicts the nestin amino acid (SEQ ID No: 2) and nucleotide (SEQ ID No:1) sequences.~~

Please replace the paragraph at pages 22, lines 19 through 28 continuing to page 23, lines 1 through 28 and page 24, lines 1 through 21, with the following replacement paragraph:

~~RT-PCR and Southern blot analysis are performed according to the following methods.~~

Total cellular RNA prepared from rat or human islets is reverse transcribed and amplified by PCR for about 35 cycles depending on the desired degree of amplification, as described previously (Daniel, et al., 1998, Endocrinology, 139:3721-3729).

Oligonucleotides used as primers or amplimers for the PCR and as probes for subsequent Southern blot hybridization are:

a9

Rat nestin:	Forward, 5'gcggggcggtgctgactac3' (SEQ ID NO: 3); Reverse, 5'aggcaagggggaagagaaggatgt3'(SEQ ID NO: 4); Hybridization, 5'aagctgaagccgaatttccttgggataccagagga3' (SEQ ID NO: 5).
Rat keratin 19:	Forward, 5'acagccagtactcaagacc3'(SEQ ID NO: 6); Reverse, 5'ctgtgtcagcacgcacgtta3'(SEQ ID NO: 7); Hybridization, 5'tggattccacaccaggcattgaccatgcca3' (SEQ ID NO: 8).
Rat NCAM:	Forward, 5'cagcgttgagagtccaaat3'(SEQ ID NO: 9); Reverse, 5'ttaactcctgtgggttgg3'(SEQ ID NO: 10); Hybridization, 5'aaaccagcagcgatctcagtggtgtggaacgatgat3'(SEQ ID NO: 11).
Rat IDX-1	Forward, 5'atcactggagcaggggaagt3'(SEQ ID NO: 12) Reverse, 5'gtactacgtttcttatct3' (SEQ ID NO: 13) Hybridization, 5'gcgtggaaaagccagtggg3'(SEQ ID NO: 14)
Human nestin:	Forward, 5'agaggggaattcctggag3'; (SEQ ID NO: 15) Reverse, 5'ctgaggaccaggactctcta3'; (SEQ ID NO: 16) Hybridization, 5'tatgaacgggctggagcagtcctgaggaaagt3'.(SEQ ID NO: 17)
Human keratin:	Forward, 5'cttttcgcgcgccagcatt3';(SEQ ID NO: 18) Reverse, 5'gattctcctgtccctcgagc3';(SEQ ID NO: 19)

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Human Glut-2

Forward, 5' gcagctgctcaactaatcac 3'(SEQ ID NO: 48)

Reverse, 5' tcagcagcacaagtcccaact 3'(SEQ ID NO: 49)

Hybridization, 5' acgggcattcttattagtcagattattggt 3'(SEQ ID NO: 50)

Human Insulin

Forward, 5' aggtctctctacaca3'(SEQ ID NO: 51)

Reverse, 5' caggctgcctgcacca 3'(SEQ ID NO: 52)

Hybridization, 5' aggcagaggacctgca 3'(SEQ ID NO: 53).

Please replace the paragraph at pages 50, lines 3 through 28 with the following replacement paragraph:

-Total cellular RNA prepared from rat or human islets was reverse transcribed and amplified by PCR for 35 cycles as described previously (Daniel et al., 1998, Endocrinology, 139:3721-3729). The oligonucleotides used as primers or amplimers for the PCR and as probes for subsequent Southern blot hybridization are:

Rat nestin:

Forward, 5' gcggggcggtgcgtgactac3' (SEQ ID NO: 3);

Reverse, 5' aggcaaggggaagagaaggatgt3' (SEQ ID NO: 4);

Hybridization, 5' aagctgaagccgaatttccttgggataccagagga3' (SEQ ID NO: 5).

Rat keratin 19:

Forward, 5' acagccagtacttcaagacc3' (SEQ ID NO: 6);

Reverse, 5' ctgtgtcagcagcacgtta3' (SEQ ID NO: 7);

Hybridization, 5' tggattccacaccaggcattgacctgcca3' (SEQ ID NO: 8).

Rat NCAM:

Forward, 5' cagcgttgagagtcctcaaat3' (SEQ ID NO: 9);

Reverse, 5' ttaactcctgtggggttg3' (SEQ ID NO: 10);

Hybridization, 5' aaaccagcagcggatctcagtggtgtggaacgatgat3' (SEQ ID NO: 11).

Rat IDX-1

Forward, 5' atcactggagcagggag3' (SEQ ID NO: 12)

Reverse, 5' gctactacgtttcttatct3' (SEQ ID NO: 13)

Hybridization, 5' gcgtggaaaagccagtggg3' (SEQ ID NO: 14)

Human nestin:

Forward, 5' agaggggaattcctggag3'; (SEQ ID NO: 15)

Reverse, 5' ctgaggaccaggactctcta3'; (SEQ ID NO: 16)

Hybridization, 5' tatgaacgggctggagcagctctgaggaaagt3'. (SEQ ID NO: 17)

Human keratin:

Forward, 5' ctttgcgcgcccagcatt3'; (SEQ ID NO: 18)